Serological evidence of human granulocytic ehrlichiosis in Switzerland

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Note

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Abstract To investigate whether human granulocytic ehrlichiosis (HGE) is prevalent in Switzerland, 1515 human serum samples from individuals with different risks for tick exposure were tested for antibodies to *Ehrlichia phagocytophila*, a surrogate marker of the agent of HGE. The distribution of titres showed marked differences between sera of individuals with no or low risk for tick exposure and those with a high risk. The results of serological testing provided evidence of HGE in Switzerland as well as evidence of two types of coinfections: those with the agent of HGE and *Borrelia burgdorferi*, and those with the agent of HGE and the central European tickborne encephalitis virus.

Key words Human granulocytic ehrlichiosis · Indirect immunofluorescent antibody test · Prevalence · Switzerland

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Introduction

Human granulocytic ehrlichiosis (HGE) is an acute, febrile disease with nonspecific symptoms, often accompanied by leukopenia, thrombocytopenia, anaemia, and a mild elevation of the transaminases [1]. Ticks of the genus *Ixodes*, which transmit *Borrelia burgdorferi* and the central European tickborne encephalitis virus, are also the likely vector of the agent of HGE. Information concerning the agent responsible for HGE and its distribution outside the USA is sparse. Epidemiological studies in Sweden [2], Norway [3], the UK [4], and northern Switzerland [5] indicate that HGE is endemic in those countries. Recently, investigators in Slovenia provided serological and molecular evidence of HGE in their country [6]. Because of the marked serological cross-reactivity between the members of the *Ehrlichia phagocytophila* genogroup, *Ehrlichia equi* and *Ehrlichia phagocytophila*, antigen can be used in serological tests for HGE [7].

In the present study, sera from 1515 individuals from five risk groups were investigated to determine the prevalence, if any, of HGE in Switzerland.

Materials and Methods

Sera from 1515 persons from five groups representing different risk categories for tick exposure were collected between January 1993 and December 1996 and tested for antibodies to *Ehrlichia phagocytophila* by indirect immunofluorescence. All persons resided in the eastern region of Switzerland.

Group 1 consisted of 373 newborn babies from which umbilical cord blood was collected, and group 2 was composed of 530 random blood donors. Group 3 consisted of 258 recreational hunters who were assumed to have a high risk of tick exposure due to their weekly hunting activities. Group 4 consisted of 149 patients who had been diagnosed previously with Lyme borreliosis by their own physicians on the basis of clinical evaluation and antibody to *Borrelia burgdorferi* screened by enzyme immunoassay (EIA) and confirmed by immunoblotting. The 205 patients in

group 5 had been diagnosed previously with acute central European tickborne encephalitis on the basis of clinical evaluation and a positive EIA test for antibodies to the central European tickborne encephalitis virus.

All serum samples were examined for antibodies to *Ehrlichia phagocytophila* (Swiss strain) by indirect immunofluorescence as described previously [8]. Values ≥ 80 were considered positive, because nonspecific reactivity is likely to occur in less diluted serum. Values from the five groups were compared using the chi-square test. P values ≤ 0.05 were considered statistically significant.

Table 1 lists the reference sera and conjugated antisera used to determine possible cross-reactivity with *Ehrlichia phagocytophila* antigen via indirect immunofluorescence. Sera from four human patients with HGE acquired in the USA (provided by J.S. Dumler, the Johns Hopkins University School of Medicine, Baltimore, USA), and sera from three horses experimentally infected with HGE (provided by J.E. Madigan, University of California, Davis, USA) were drawn during the acute phase of infection and again approximately 30 days later.

Results and Discussion

Our approach was to examine groups of individuals with different risk factors for tick exposure. Group 1 comprised newborns, chosen because tick exposure can be safely ruled out in these subjects; 99.5% of the sera from this group had a negative antibody titre (Table 2). Two samples had titres of 80, which may have reflected maternal antibodies. Unfortunately, maternal serum samples were not available to confirm our suspicion. In comparison, 0.85% of babies born to mothers living in Switzerland had positive titres for *Borrelia burgdorferi* [9]. Group 2, consisting of randomly chosen blood donors, was presumed to represent individuals with a relatively low, or average, risk of tick exposure; however, because the donors remained anonymous, the true exposure rate was unknown. Of the samples from this group, 98.9% had a titre of <80, and only six (1.1%) had a titre of 80. Group 3 comprised hunters, who were chosen because they, by virtue of their recreational activity, can be expected to have a higher risk of tick bites compared with the average population. This expectation was borne out by a statistically significant higher incidence of positive titres (9%) in this group as compared with groups 1 and 2 ($p<0.001$).

As expected, the highest seroprevalence occurred in individuals seropositive for *Borrelia burgdorferi* (12.7%) or the central European tickborne encephalitis virus (19.5%). Compared with groups 1 and 2, these differ-

Table 1 Reference sera and conjugated antisera used to determine specificity

Reference serum	Species	Conjugated antiserum
Coxiella burnetti[a]	bovine	FITC conjugated rabbit anti-bovine IgG[c]
Ehrlichia canis[a]	canine	FITC conjugated rabbit anti-dog IgG[d]
Ehrlichia risticii[a]	equine	FITC conjugated goat anti-horse IgG[e]
Ehrlichia chaffeensis[b]	human	FITC conjugated goat anti-human IgG[f]
Rickettsia rickettsii[b]	human	FITC conjugated goat anti-human IgG[f]
Rickettsia typhi[b]	human	FITC conjugated goat anti-human IgG[f]
Brucella abortus[a]	bovine	FITC conjugated rabbit anti-bovine IgG[c]
Anaplasma marginale[a]	bovine	FITC conjugated rabbit anti-bovine IgG[c]
Babesia bovis[a]	bovine	FITC conjugated rabbit anti-bovine IgG[c]
Babesia divergens[a]	bovine	FITC conjugated rabbit anti-bovine IgG[c]

[a] Reference sera were obtained from the U.S. National Veterinary Services Laboratories (Ames, Iowa)
[b] Reference sera obtained from J. S. Dumler, the Johns Hopkins University School of Medicine (Baltimore, Maryland)
[c] Rabbit anti-bovine/FITC, Nordic Immunological Laboratories b.v., The Netherlands
[d] Fluorescein-conjugated affinipure rabbit anti-dog IgG, Jackson ImmunoResearch Laboratories, Inc., USA
[e] Goat anti-horse IgG affin. FITC-conjugated, Chemical Credential, USA
[f] Fluorescein-conjugated affinipure goat anti-human IgG, Jackson ImmunoResearch Laboratories, Inc., USA

Table 2 Distribution of titres to *Ehrlichia phagocytophila* in five groups of individuals with different risk categories for tick exposure

Group (no. of samples)	No. (%)		
	Titer <80	Titer $=80$	Titer $=160$
Newborns (373)	371 (99.5)	2 (0.5)	0
Blood donors (530)	524 (98.9)	6 (1.1)	0
Hunters (258)	235 (91.0)	19 (7.4)	4 (1.6)
Patients with Lyme borreliosis (149)	130 (87.3)	13 (8.7)	6 (4.0)
Patients with CETE (205)	165 (80.5)	35 (17.1)	5 (2.4)
Total (1515)	1425 (94.0)	75 (5.0)	15 (1.0)

CETE, central European tickborne encephalitis

ences were statistically significant ($p < 0.001$). Other investigators from Norway [3] and Switzerland [5] have studied patients with Lyme disease for antibodies to the *Ehrlichia equi* antigen, and found a comparable seroprevalence of 10.2% and 17.1%, respectively. Unlike *Borrelia burgdorferi*, the virus causing central European tickborne encephalitis has a markedly heterogeneous geographic distribution in Switzerland and occurs predominantly in so-called natural foci [10]. This distribution may explain the low discrepancy between the number of positive serum samples in groups 4 and 5.

Reference sera used for determination of cross-reactivity had antibody titres to *Ehrlichia phagocytophila* of 10 or 20; the exception was *Ehrlichia chaffeensis* seropositive reference sera, which had a titre to *Ehrlichia phagocytophila* of 40. All acute and postinfection sera with documented acute HGE infection showed seroconversion: a two- to four-fold increase in titres was observed in sera from humans, and a four- to seven-fold increase in titres was found in sera from equines.

Of the four human patients with HGE from whom paired sera were collected, two had titres of 40 thirty days after the acute phase. According to the criteria described, these results would have been classified as negative. The minimal increase in titre in these two patients might be explained by the relatively rapid recovery of these individuals following antibiotic treatment and the fact that a decreased bacterial load may have favoured a low seroresponse. Another possible explanation is that antigenic structural diversity could exist among otherwise indistinguishable granulocytic ehrlichial isolates. Nevertheless, we assumed that the specificity and sensitivity of our anti-*Ehrlichia phagocytophila* antibody test was acceptable, and thus, the *Ehrlichia phagocytophila* antigen was suitable for detection of serum antibodies to the agent of HGE acquired in Europe.

We realise that findings from serological examinations to detect an infection that has not yet been identified in Switzerland by clinical observations, by direct detection of the organism, or by molecular techniques, must be interpreted with caution. However, several observations indicate that the occurrence of HGE in Switzerland is possible and, in fact, very likely. First, the probable vector for the agent of HGE, *Ixodes ricinus*, has a wide distribution in Switzerland and, depending on the region, may transmit *Borrelia burgdorferi* and/or the central European tickborne encephalitis virus. Second, we recently reported a new subspecies of the *Ehrlichia phagocytophila* genogroup in dogs in Switzerland [11]; of the 16S rRNA gene this subspecies had a 100% homology with that of the agent of HGE [12].

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